## Remarks

The Applicants have amended various of the claims as described below to place them into final condition for allowance.

Claims 13, 17, 21, 26, 28 and 30 have been simplified to a peptide "consisting of SEQ ID NO: 2" instead of "consisting of the sequence shown in SEQ ID NO: 2" and the repetition of the expression "consisting of SEQ ID NO: 2" within the claims has been deleted.

Claims 17 and 21 have been amended to recite a method drawn to "treating a mammal with melanoma" instead of "treating melanoma in a mammal in need thereof."

Claims 25, 27 and 29 have been simplified to recite that the "sequence consists of SEQ ID NO: 1" instead of that the "sequence encoding the disintegrin domain consisting of the sequence shown in SEQ ID NO: 2 consists of the sequence shown in SEQ ID NO: 1."

Claims 13, 17, 21, 26, 28 and 30 have been amended to recite that the expression plasmid is administrated by "intramuscular or intratumoral injection, followed by application of electric pulses to a corresponding intramuscular or intratumoral injected site(s)" instead of by "direct inoculation and electrotransfer to an intramuscular site, or an intratumoral site."

Claims 13, 17 and 21 have been amended to recite a "plasmid coding for a therapeutic peptide consisting of SEQ ID NO: 2 absent any operably linked coding sequence" instead of a "plasmid coding for the disintegrin domain consisting of SEQ ID NO: 2." Moreover, Claims 13, 17, 21, 26, 28 and 30 now recite "the therapeutic peptide" instead of "the disintegrin domain."

Entry of the above changes into the official file is respectfully requested.

Claims 13, 17, 21 and 25-30 stand objected to with respect to the portion of the claims surrounding SEQ ID NO: 2. The Applicants have amended Claims 13, 17 and 21 as noted above. The Applicants respectfully submit that those amendments address the objection and therefore render it moot. The Applicants have also amended Claims 25, 27 and 29, thereby rendering that objection moot as well. Withdrawal of the objection is respectfully requested.

Claims 13, 17, 21 and 25-30 stand rejected under 35 USC §112 as not being enabled. The Applicants note with appreciation the Examiner's helpful comments with respect to enablement. The Applicants provide the following comments and respectfully submit that, when taken with the above mentioned claim amendments, render all of the claims fully enabled.

Those comments are broken into three groups as noted below, namely, route of administration, site of administration and lack of *in vitro* clinical trials.

Page 3 of the rejection acknowledges that Claims 13, 17, 21 and 25-30 are enabled for "a method of direct injection to a melanoma or a pulmonary metastasis of a nucleic acid consisting of SEQ ID NO: 1 operably linked to an expression control sequence, followed by application of electric field pulses to the melanoma or the pulmonary metastasis" resulting in "the decrease in the number of intratumoral vessels and in inhibition of growth of the melanoma or inhibition of the pulmonary metastases." However, the rejection maintains that the specification does not enable other methods.

Claims 13, 17, 21, 26, 28 and 30 have been amended as noted above to recite that the expression plasmid is administered by "intramuscular or intratumoral injection, followed by application of electric pulses to a corresponding intramuscular or intratumoral injected site(s)."

Page 3 of the rejection further states that "the specification teaches that the disintegrin domain of metargidin when delivered to a tumor, or metastases site can cause a diminution of vessels and thus lead to a decrease in pulmonary metastases and melanoma growth." However, the rejection considers that it is "not clear what relationship the intratumoral site or intramuscular site have to the intratumoral vessels."

First, the Applicants note that the disintegrin domain of metargidin (AMEP) may act in trans. This means that it may be produced by one cell and exert its function on another cell. Indeed, as shown in Examples 3 and 7, it is thought that AMEP principally acts through the binding of integrin alpha v beta 3 (paragraphs [0112] and [0127] of the application as published). Integrin alpha v beta 3 is a trans-membrane protein expressed on the outer surface of the endothelial cells of blood vessels. Therefore, integrin alpha v beta 3 is in contact with blood. Thus, to interact with the outer domain of integrin alpha v beta 3, the AMEP molecule only needs to be present in sufficient concentration in circulating blood.

As shown in Trochon-Joseph *et al.* 2004 (*Cancer Research* 64, 2062-2069), electrotransfer of a plasmid bearing the AMEP gene into mouse skeletal muscles *in vivo* leads to rapid, stable and tightly regulated expression of the transgene. Moreover, the AMEP protein can be detected in blood (at a concentration of ~50 ng/ml) for more than 2 months (page 2068, second paragraph). Therefore, the tumoral or muscular cell which incorporates the expression

plasmid injected in the tumor or muscle, respectively, may only be considered as a production cell that synthesizes and secretes the therapeutic peptide. Once secreted, the peptide may interact with the cell that has just produced it. But it may also be transported by blood and reach another cell belonging to another tissue or another site of the body. In this case, the muscle may for instance be used as a site of production of the therapeutic peptide. Then, the peptide may be transported by blood to a tumoral site or to metastases where it exerts inhibitory functions.

Finally, the results disclosed on page 25 of the Applicants' specification show that the method is efficient either when the nucleic acid is delivered to the target site, or when it is delivered to a site different from the target site:

a) INHIBITION OF TUMOR GROWTH BY AMEP:

To that end, a plasmid encoding AMEP was injected and electrotransfered in the tibial cranial muscle of mice (paragraphs [0087] and [0072]). The tibial cranial muscle is a muscle inserted on the tibia bone of mice.

Mice were then injected subcutaneously in the back with MDA-MB-231 cells, and the tumors were let to grow until reaching a volume of 18 $mm^3$. Then, doxycycline was added to the mice's drinking water to induce expression of AMEP (*i.e.*, the polypeptide of sequence SEQ ID NO: 2) in the muscles of the mice (paragraph [0073]). The results show that expression of AMEP led to markedly smaller tumor volumes as compared with control, and to significant inhibition of the number of vessels in the tumors (paragraph [0088], Fig. 8 and table 3).

Accordingly, these results demonstrate that injection followed by electrotransfer of a plasmid encoding AMEP in a tibia muscle of mice and expression of AMEP in the muscle inhibits growth and vascularisation of tumors implanted in the back of the mice. Hence, these results demonstrate efficacy of the claimed methods although the nucleic acid is injected and electrotransfered to a site different from the target site (muscle *vs.* tumor site).

b) INHIBITION OF THE FORMATION OF PULMONARY METASTASES BY AMEP:

To that end, C57B1/6 mice were used which were previously injected with a plasmid encoding AMEP into the tibial cranial muscle followed by electrotransfer (paragraph [0072]). The mice were further injected with Bl6F10 mouse melanoma cells in the retro-orbital sinus, to

establish pulmonary metastases. Three days after this injection of B16F10 cells, AMEP expression in the tibial cranial muscle of the mice was induced by adding doxycycline to the drinking water of the mice (paragraph [0076]).

The results show that production of AMEP in the muscle of the mice inhibited the number of pulmonary metastases (paragraph [0091] and Figs. 9 and 10). Accordingly, these results demonstrate efficacy of the claimed methods although the nucleic acid is injected and electrotransfered to a site different from the target site (muscle *vs.* pulmonary metastases).

Similarly, the data provided in the Declaration of Dr. Trochon-Joseph dated May 8, 2007, related to the inhibition of the growth of B16F10 and C9 melanoma tumors implanted in the back of mice which were injected intratumorally (*i.e.*, in the melanoma) with plasmids encoding AMEP and electrotransfered. The results provided showed that intratumoral injection followed by electrotransfer of a plasmid encoding AMEP achieved inhibition of melanoma growth. Furthermore, the data provided in the Declaration of Dr. Trochon-Joseph dated January 5, 2009 also illustrate that intratumoral injection followed by electrotransfer of a plasmid encoding AMEP inhibited growth of B16F10 or C9 melanoma in mice. In particular, inhibition of the growth of B16F10 and C9 melanoma was correlated with a reduction in the number of tumor blood vessels.

Altogether, the results provided in the application as filed (under oath) and in the Declarations executed by Dr. Trochon-Joseph demonstrate that:

- reduction of the number or formation of tumor vessels can be achieved by both intramuscular or intratumoral electrotransfer of a plasmid encoding AMEP,
- inhibition of melanoma growth can be achieved by both intramuscular or intratumoral electrotransfer of a plasmid encoding AMEP, and
- inhibition of pulmonary metastases can be achieved by intramuscular electrotransfer of a plasmid encoding AMEP.

The Applicants have thus demonstrated that there is no need for the claimed methods to work that the plasmid encoding AMEP be administered to the very specific target site where the effects of the AMEP peptide are expected to operate (*i.e.*, the melanoma or pulmonary metastases).

Actually, expression of AMEP in the tibial cranial muscle, *i.e.*, a muscle inserted on the tibia bone which is remote from the target sites (back of the mice for the implanted B16F10 and C9 melanoma, and lungs for the pulmonary metastases), has been shown to efficiently reduce the number or formation of tumor vessels, as well as inhibit the growth of melanoma tumors and development of pulmonary metastases. Therefore, the Applicants respectfully submit that there is no lack of clarity in the relationship between the intratumoral or intramuscular site of administration and the targeted pulmonary metastases or melanoma.

LACK OF *IN VIVO* CLINICAL TRIALS

The rejection also contends that animal models are not well correlated with *in vivo* clinical trial results in patients. However, the rejection also admits that "human trials are not required but means of enabled treatment are required." The Applicants respectfully submit that the means of treatment recited in the claims, *i.e.*, intratumoral or intramuscular electrotransfer of a plasmid encoding AMEP, have been thoroughly demonstrated to successfully achieve inhibition of number of formation of intratumoral vessels, prevention and/or treatment of melanoma and prevention and/or treatment of pulmonary metastases as recited in each of Claims 13, 17 and 21.

Furthermore, parts of these results were obtained with melanoma established with cell lines of human origin, *i.e.*, MDA-MB-231 cells and C9 cells.

Furthermore, preclinical trials have shown that electrotransfer of a plasmid encoding AMEP leads to higher tumor growth inhibition than temozolomide, a melanoma reference treatment. This is demonstrated in the enclosed Declaration of Dr. Trachon.

Recently, the Health Authorities of Denmark and Slovenia have authorized the establishment of phase I clinical trial for the treatment of advanced and metastatic melanoma by AMEP as shown in the enclosed excerpt from the website ClinicalTrials.gov. Thus the results obtained in preclinical trials are believed be confidently predictive of results in humans by Health Authorities.

The Applicants have accordingly amended the claims and submitted multiple Declarations demonstrating enablement in accordance with the scope of those claims. Those established facts, taken with the experimental results provided in the application originally submitted under oath, readily satisfy §112. As acknowledged in the rejection, the Applicants are

in no way required to provide clinical trials or data as evidenced in clinical trials and even referring to such clinical trials in a rejection is irrelevant because of the lack of requirement for such results. The Applicants have otherwise provided substantial evidence on the record establishing enablement. Withdrawal of the rejection is respectfully requested.

Claims 13, 17, 21 and 25-30 stand rejected under 35 USC §103 over the hypothetical combination of Merkulov and Fanslow with Bettan. The Applicants again note with appreciation the Examiner's detailed comments hypothetically applying the combination against those claims. The Applicants nonetheless respectfully submit that the combination fails to disclose, teach or suggest the subject matter of those claims. In addressing the rejection, the Applicants will first provide individual comments concerning the deficiencies of the three references. Then, the Applicants will provide further comments demonstrating why the combination would not be made and that, in any event, one skilled in the art would not have a reasonable expectation of success in making that combination.

Merkulov discloses a protein having protease activity which amino acid sequence comprises the disintegrin domain of sequence SEQ ID NO: 2. However, Merkulov does not disclose, teach or suggest expressing a plasmid coding for a therapeutic peptide consisting of SEQ ID NO: 2 absent any operably linked coding sequences, as recited in Claims 13, 17 and 21, to decrease the number or formation of intratumoral vessels, or to treat and/or prevent melanoma or pulmonary metastases.

According to the rejection, "Bettan et al. speak to the success of intramuscular administration in mammals." However, the rejection acknowledges that "Bettan et al. do not speak to the nature of the gene to be introduced." Bettan discloses intratumoral electrotransfer of plasmids encoding the luciferase or β-galactosidase reporter gene. Bettan merely teach that "electrotransfer leads to increase number of transfected cells [...] [and] to an increase in the number of plasmids entering each transfected cells" (sentence spanning pages 87 and 88). The authors conclude that "electrotransfer technology could be applied with therapeutic genes to treat accessible tumors" (page 89, col. 1, end of last but one paragraph).

First, the Applicants respectfully submit that the teaching of Bettan only refers to the treatment of accessible tumors. Therefore, it does not provide any teaching or guidance about the

use of electrotransfer technology for the treatment of metastasis, or vessels which would not be accessible.

Moreover, the Applicants invite the Examiner's attention to the fact that electrotransfer of a given gene (here the luciferase or the β-galactosidase reporter gene) leads to efficient transfection of cells does not mean that electrotransfer of another gene (here the AMEP gene) will lead to efficient transfection of cells. Indeed, the efficiency of the delivery of a gene into a cell by transfection depends on many factors including the length of the gene, the nature of the plasmid in which it is incorporated, the presence of secondary structures.... Therefore, the teaching of Bettan related to the success of intramuscular electrotransfer of the luciferase and the β-galactosidase reporter genes cannot be reasonably extrapolated to any other gene.

However, the data provided in the Applicants' specification and the Declarations of Dr. Trochon-Joseph demonstrate that injection followed by electrotransfer was successfully used to deliver several types of plasmids encoding AMEP (in particular vector pBi (paragraph [0071] of the application and Declarations dated May 8, 2007 and January 5, 2009); vector pVAX1 (Declarations dated May 8, 2007 and January 5, 2009); and vector pORT (Declaration dated January 5, 2009)), both intramuscularly and intratumorally.

Also, the proteins produced from the luciferase and the β-galactosidase reporter genes are strictly cytoplasmic proteins. In particular, they are not secreted out of the cell, once produced. To the contrary, the Applicants' therapeutic peptide is secreted by the cell after synthesis. The fact that electrotransfer of a given gene allows efficient transfection of cells and efficient production of the corresponding peptide in the cytoplasm of the cell does not necessarily mean that the peptide will be correctly secreted. Indeed, to be secreted, the neo-synthesized peptide has to be well conformed and follow specific routes in the cell leading to its secretion, subsequently allow for its detection in blood.

Nothing in Bettan discloses, teaches or suggests that electrotransfer of a given gene coding for a secreted peptide could lead to the correct secretion of that peptide. Therefore, Bettan does not teach or suggest that the AMEP peptide can be efficiently produced and secreted following electrotransfer into a cell. Bettan does not either teach or suggest that electrotransfer

of a therapeutic gene may be used for the treatment of metastasis or vessels which would not be accessible.

The rejection considers that "Fanslow *et al.* teach that disintegrin domains from a variety of Adam proteins such as metargidin can be used to inhibit angiogenesis and endothelial cell migration." However, the rejection acknowledges that "Fanslow *et al.* do not provide the sequences used." The Applicants invite the Examiner's attention to the fact that Fanslow discloses that ADAM desintegrin domains in general are useful for inhibiting the biological activity of integrins and for inhibiting endothelial cell migration and angiogenesis (col. 3, lines 14-18). Fanslow lists in particular a group of ten ADAM disintegrin domains as possible embodiments of the invention they described, including ADAM-15 disintegrin domain, *i.e.*, the disintegrin domain of metargidin.

Fanslow characterized ADAM-15 disintegrin domain fused to the constant fragment (Fc) of an immunoglobulin (ADAM-15 dis-Fc) as binding to integrins αvβ3 and α5β1 in Table 3 and col. 17, lines 9-10 and 11-12. The rejection states that Fanslow recognizes that the disintegrin domain alone can be used. However, the Applicants respectfully submit that the polypeptide which was actually assayed by Fanslow is not a polypeptide which is a disintegrin domain consisting of SEQ ID NO: 2.

Moreover, there was a prevailing prejudice in the art according to which the disintegrin domain of adamalysin alone would not be stable. In support to this assertion, the Applicants invite the Examiner's attention to Nath (1999, *Journal of Cell Science*, 112, 579-587). Nath teaches "the disintegrin domain alone may not be stable on its own, due to the presence of an odd number of cysteine residues, which may be part of an inter-domain disulphide bond or may be involved in receptor oligomerisation" (Jia *et al.*, 1997) at page 581, col. 2, paragraph under the heading "Cell-chimeric adhesion assays for metargidin binding."

Moreover, the disintegrin domain is a cysteine-rich domain which contains 15 cysteines. Usually, cysteine-rich polypeptides do not fold properly and fusing them to long polypeptides (such as Fc) is a well-known way to allow proper folding of the polypeptide. Therefore, in Fanslow, all assayed ADAM disintegrin domains were provided as fusion polypeptides with the Fc part of an immunoglobulin as seen in. *e.g.*, col. 15, Table 2 of Fanslow. Fanslow teaches that "certain polypeptides derived from antibodies are among the peptides that can promote

multimerization of ADAM disintegrin domain polypeptides attached thereto" (col. 9, lines 28-31). The Fc part of an immunoglobulin is one of such polypeptides promoting multimerization. Fanslow actually describes that two polypeptides consisting of ADAM disintegrin domain fused to an Fc polypeptide form a dimer (col. 9, lines 44-47).

Accordingly, Fanslow did not assay the activity of an ADAM disintegrin domain alone, but the activity of ADAM disintegrin multimers in which the disintegrin domain is fused to the Fc fragment of an immunoglobulin. To the contrary, the Applicants administered an expression plasmid coding for the disintegrin domain to a mammal. This leads to expression of the disintegrin domain *in vivo*. The therapeutic effects observed following such administration imply that the disintegrin domain thus produced *in vivo* displays proper folding allowing its function.

Altogether, the teaching of Fanslow would not have led one skilled in the art to defy the prejudice prevailing in the art according to which the disintegrin domain alone is not stable. Accordingly, the assertion in the rejection that known products (the disintegrin domain of an ADAM) would provide "predictable results" is not substantiated.

The rejection considers that: "it is obvious to combine known technologies with known products for predictable results and Bettan et al. teach that it is known to administer treatment modalities on expression vectors encoding the product by electrotransfer and Merkulov and Fanslow teach that disintegrin domain provide successful modalities." The Applicants disagree.

The Applicants respectfully submit that:

(i) the teaching of Fanslow and Merkulov would not have led one skilled in the art to select a gene encoding the polypeptide consisting of sequence SEQ ID NO: 2 for electrotransfer, and

(ii) there was no reasonable expectation that expression of the polypeptide of sequence SEQ ID NO: 2 in the muscle or the tumor would lead to inhibition of the number or formation of tumor vessels, or to the treatment and/or prevention of melanoma or pulmonary metastases.

Accordingly, the combination of Bettan with Fanslow and Merkulov:

(i) would not have lead one skilled in the art to select a gene encoding a disintegrin domain consisting of SEQ ID NO: 2 for administration by electrotransfer because their collective teachings would not have caused one skilled in the art to defy the prejudice prevailing in the art according to which the disintegrin domain alone would be instable;

(ii) would have led, at best, one skilled in the art to express an ADAM-20 or 23 disintegrin domain fused to the Fc part of an immunoglobulin rather than ADAM-15 disintegrin domain fused to the Fc part of an immunoglobulin;

(iii) but would have given no clue to one skilled in the art that *in vivo* expression of the disintegrin domain of an ADAM, not even speaking of the disintegrin domain of metargidin (ADAM-15) of sequence SEQ ID NO: 2, could successfully result in the claimed subject matter.

The Applicants accordingly respectfully submit that one skilled in the art would not make the hypothetical combination but, in any event, one skilled in the art would have no reasonable expectation of success in making such a combination. Withdrawal of the rejection is respectfully requested.

In light of the foregoing, the Applicants respectfully submit that the entire application is now in condition for allowance, which is respectfully requested.

Respectfully submitted,

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